

Postpolymerization Synthesis of (Bis)Amide (Co)Polymers: Thermoresponsive Behavior and Self-association

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Abstract. A library of novel well-defined (bis)amide-based (co)polymers was prepared through postpolymerization modification of poly(pentafluorophenyl acrylate) with amines including ammonia and amide derivatives of amino acids. Products were characterized using a combination of NMR and FT-IR spectroscopies and size-exclusion chromatography; results conformed to the expected structures obtained through quantitative conversion. The series of (bis)amide (co)polymers displayed rich phase behavior in aqueous solution such as thermoreversible gelation at low temperature and high concentration while other samples displayed inverse temperature dependent solubility (lower critical solution temperature (LCST)-type) behavior. A hydrophobically modified polyacrylamide copolymer displayed upper critical solution temperature (UCST) behavior in aqueous solution. Significantly, driven by polymer–polymer hydrogen bonding, copolymers self-associated into highly ordered, regular structures of several tens to hundreds of micrometers in size. Morphologies included sheet-like, rod-like and honeycomb-like structures and depended strongly on the chemical composition of copolymers.

Introduction

Several polyacrylamide species comprised of *N*-acryloyl derivatives of amino acid amides have recently become the focus of intense research in different fields of polymer science. Featuring two (or more) amide groups in each monomer segment, each acting as hydrogen bond donor and acceptor, the behavior of these hydrophilic polymers is primarily dictated by strong intra- and intermolecular polymer–polymer interactions. Depending on temperature these attractions can outweigh polymer–water interactions in aqueous solution. The simplest amino acid amide-based polyacrylamide, poly(*N*-acryloyl glycinamide) (pGlyAm), for example, exhibits thermally reversible protein-like gelation in water and physiological buffer solution at low temperature due to significant hydrogen bonding interactions.[1, 2] Moreover, pGlyAm, poly(*N*-acryloyl asparaginamide) (pAspAm), and poly(*N*-acryloyl glutaminamide) (pGlnAm) were recently reported by Ohnishi's group,[3-5] and others,[6, 7] to exhibit upper critical solution temperature (UCST) behavior in water—a rare phenomenon observed only for a few thermoresponsive (co)polymers.[8, 9] Research in Agarwal's group revealed that the behavior of pGlyAm depends strongly on the synthetic procedure used to produce this species, with UCST transitions occurring only if rigorous care is taken to avoid incorporation of any traces of ionic groups into the polymer.[10] With appropriate synthetic care however, pGlyAm exhibiting sharp reproducible UCST transitions is accessible.[11, 12] Similarly, hydrophobically modified polyacrylamido species have been shown to exhibit UCST transitions in water, likewise governed by inter-polymer hydrogen bonding between amide groups.[13]

Postpolymerization modification[14-18] is a versatile technique for the synthesis of functional (co)polymers and has lent itself perfectly to the investigation of structure-property relationships in thermoresponsive materials since libraries of homopolymers and statistical copolymers with identical average degrees of polymerization and controllable molar compositions can easily be prepared.[19-28] Of a wide variety of stimulus responsive

(co)polymers prepared through postpolymerization modification techniques, only very few (co)polymer species exhibit aqueous UCST behavior. Examples include ureido-functional copolymers[29, 30] and zwitterionic polysulfobetaines.[31] Activated esters such as pentafluorophenyl (meth)acrylate (PFP(M)A) constitute a very versatile class of reactive scaffold.[18] Exhibiting highly selective reactivity toward primary and secondary amines, PFP(M)A polymers (pPFP(M)A) provide simple access to functional poly(*N*-alkyl (meth)acrylamide)s and poly(*N,N*-bisalkyl (meth)acrylamide)s including several well-investigated lower critical solution temperature (LCST)-type thermoresponsive species, e.g. poly(*N*-isopropylacrylamide) (pNIPAM).[24]

In this study we investigated the modification of reactive pPFPA scaffolds as a route to (bis)amide functional polymeric species including hydrophobically modified polyacrylamides, pGlyAm, and several novel amino acid amide-derived (co)polymers. We took advantage of the postpolymerization concept to produce a range of homopolymers and statistical copolymers with an initial goal of probing aqueous solution behavior and identifying novel thermoresponsive (co)polymers. While UCST behavior was found for only one sample, several (co)polymers showed thermoreversible gelation or LCST transitions in water. Additionally, due to their strong hydrogen-bonding capacity, we found (co)polymers able to self-associate into tubular or sheet-like structures in the dry state, offering a novel approach to well-defined self-assembled morphologies.

Experimental Section

Instrumentation

NMR spectroscopic measurements were performed on a Bruker Avance 300 MHz instrument.

Internal solvent signals were used as reference: $\delta(\text{DMSO-d}_6) = 2.50$ ppm, $\delta(\text{D}_2\text{O}) = 4.79$ ppm.

Size exclusion chromatography (SEC) was performed on a Shimadzu system equipped with four 300×7.8 mm² linear phenogel columns (10^5 , 10^4 , 10^3 and 500 Å) operating at a flow rate of 1 mL/min using dimethylacetamide as eluent. The system was calibrated with a series of narrow molar mass distribution polystyrene standards with molar masses ranging from 0.58–1820 kg/mol.

Fourier transform infrared (FT-IR) spectroscopy was performed on a Bruker IFS 66/S instrument under attenuated total reflectance (ATR) and data was analyzed on OPUS software version 4.0.

Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano ZS at a scattering angle of 173° and were analyzed by Malvern Zetasizer Software version 6.20.

Turbidity measurements were performed on a Varian Cary 300 Scan spectrophotometer equipped with a Cary temperature controller and a Peltier heating element in quartz cuvettes of 10 mm path length at a wavelength of 520 nm with heating / cooling rates of $1^\circ\text{C}/\text{min}$. Polymer concentrations were 5 g/L. For clear solutions the baseline was corrected to zero absorbance, A . Transmittance, $T = 10^{-A}$, was plotted against temperature and cloud points were determined at $T = 50\%$.

Scanning electron microscopy (SEM) was conducted on a FEI Nova NanoSEM 230 using an Everhart-Thornley (ET) detector and an accelerating voltage of 5 kV. Samples were freeze-dried from aqueous solution (concentration = 2 g/L), carefully deposited on a sticky carbon film and plasma-coated with a thin layer of chromium.

Materials and Synthesis

Amide derivatives of amino acids glycineamide·HCl (**1**), L-alanineamide·HCl (**2**), L-serineamide·HCl (**3**), L- α -aminobutyramide·HCl (**4**), L-valineamide·HCl (**5**), L-leucineamide·HCl (**6**), β -alanineamide·HCl (**7**), and the dipeptide glycylglycine (**8**), were purchased from Chem-Impex International. All other reagents were purchased from Sigma-Aldrich and used as received. Pentafluorophenyl acrylate was prepared as published elsewhere.[31]

Poly(pentafluorophenyl acrylate), pPFPA, with two different molar masses was prepared by the RAFT process using benzylpropyltrithiocarbonate as chain transfer agent in analogy to a literature procedure.[31] pPFPA1, $M_{n,theor.}$ (from conversion) = 24.7 kg/mol., $DP_{theor.}$ = 103, $M_{n,SEC}$ = 46.8 kg/mol (PS standard), $\bar{D}_M = M_{w,SEC}/M_{n,SEC}$ = 1.35; pPFPA2, $M_{n,theor.}$ (from conversion) = 9.5 kg/mol., $DP_{theor.}$ = 39, $M_{n,SEC}$ = 19.8 kg/mol (PS standard), $\bar{D}_M = M_{w,SEC}/M_{n,SEC}$ = 1.40. ^{19}F NMR (290 MHz, $CDCl_3$), δ/ppm = -153.2 (bm, 2 F, *ortho*), -157.1 (bs, 1 F, *para*), -162.4 (bs, 2 F, *meta*).

Postpolymerization Modification of pPFPA with Amines: General Procedure. pPFPA (166.6 mg, 0.7 mmol of repeat units) was dissolved in anhydrous *N,N*-dimethylformamide (DMF, 2 mL) and acrylamide (4 mg, 0.056 mmol) (to scavenge thiol groups generated by RAFT end group cleavage) was then added. In a separate vial an amine (see table 1) (1.75 mmol, 2.5 equiv.) was dissolved in DMF (1 mL). For amines used as hydrochloride salts, 1,8-diazabicycloundec-7-ene (DBU, 0.419 mL, 2.8 mmol, 4 equiv.) was added. The amine solution was then quickly added into the polymer solution and the mixture stirred at room temperature overnight. Copolymers were prepared by an analogous protocol using a mixture of two different amines (total amount 1.05 mmol, 1.5 equiv). For example, for a reaction containing a feed of 50% of alanineamide and 50% of glycineamide, 0.75 equiv of L-alanineamide·HCl and 0.75 equiv of glycineamide·HCl were mixed with DBU in DMF, and then introduced into a solution containing 1 equiv. of PFPA groups. Product polymers were purified by dialysis against water in regenerated cellulose membranes with a molecular

weight cut-off of 3500 g/mol for 3 days with solvent changes 3 times a day, followed by freeze-drying. Yields ranged between 83 and 91 %. GlyAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 3.65$ ($-\text{NHCH}_2-$). AlaAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 4.21$ ($-\text{NHCH}$), 1.25 ($-\text{CH}_3$). SerAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 4.96$, 4.34 ($-\text{CH}_2\text{OH}$), 3.66 ($-\text{NHCH}$). AbuAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 4.16$ ($-\text{NHCH}$), 1.58 ($-\text{CH}_2\text{CH}_3$), 0.88 ($-\text{CH}_2\text{CH}_3$). ValAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 4.10$ ($-\text{NHCH}$), 2.44 ($-\text{CH}(\text{CH}_3)_2$), 0.87 ($-\text{CH}(\text{CH}_3)_2$). LeuAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 4.16$ ($-\text{NHCH}$), 2.43 ($-\text{CH}_2\text{CH}(\text{CH}_3)_2$), 1.58 ($-\text{CH}_2\text{CH}(\text{CH}_3)_2$), 0.88 ($-\text{CH}_2\text{CH}(\text{CH}_3)_2$). β -AlaAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 3.56$ ($-\text{NHCH}_2\text{CH}_2-$), 2.66 ($-\text{NHCH}_2\text{CH}_2-$). GlyGlyAm (co)monomer units, ^1H NMR (D_2O , 300 MHz), $\delta/\text{ppm} = 4.22$ ($-\text{NHCH}_2\text{COOH}$), 3.63 ($-\text{NHCH}_2\text{CONH}-$). BzAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 7.33$, 7.26 (m, $-\text{CH}_2\text{C}_6\text{H}_5$), 4.29 ($-\text{CH}_2\text{C}_6\text{H}_5$). HexAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 3.01$ ($-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.40 ($-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.25 ($-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.88 ($-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$). DEGA (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 3.52$, 3.45 ($-\text{NHCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_3$), 3.25 ($-\text{NHCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_3$). PentAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 3.01$ ($-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.40 ($-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.24 ($-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.87 ($-\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$). IPAm (co)monomer units, ^1H NMR (DMSO- d_6 , 300 MHz), $\delta/\text{ppm} = 3.84$ ($-\text{NHCH}(\text{CH}_3)_2$), 1.04 ($-\text{NHCH}(\text{CH}_3)_2$).

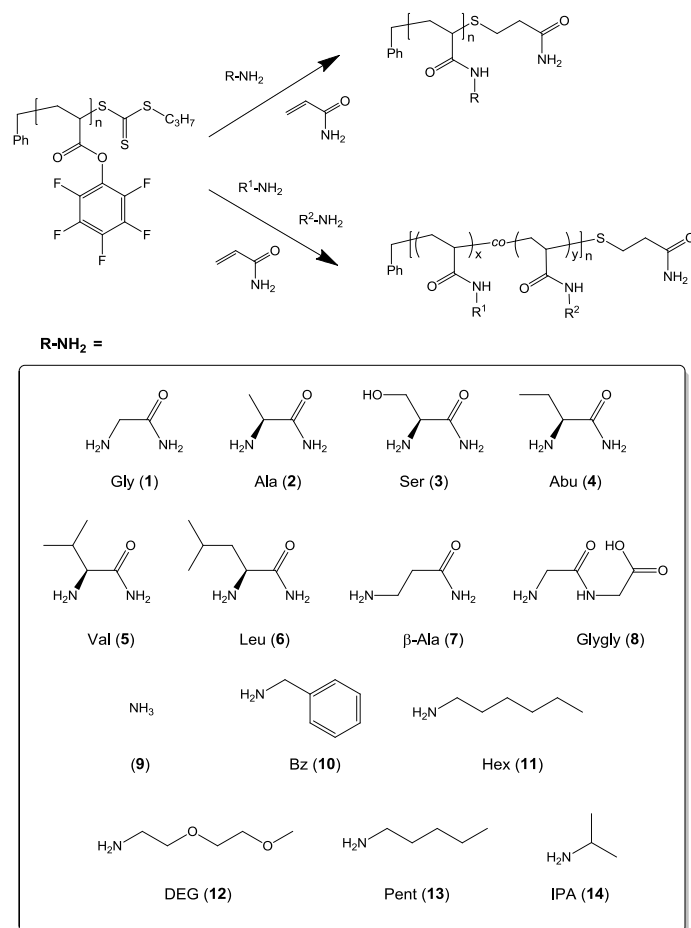
Results and Discussion

Synthesis

Postpolymerization modification of pPFPA precursors successfully yielded a library of well-defined amino acid amide-functional bisamide (co)polymers. Two reactive precursors, pPFPA1 ($M_{n,theor.} = 24.7$ kg/mol, $D_M = 1.35$) and pPFPA2 ($M_{n,theor.} = 9.5$ kg/mol, $D_M = 1.40$), prepared by the RAFT process,[32] were reacted in DMF with amide-functional amines and amine mixtures, producing bisamide homo- and copolymers, which were purified by dialysis against ultrapure water. The selection of amines comprised amide derivatives of the amino acids glycine (Gly), alanine (Ala), serine (Ser), valine (Val), and leucine (Leu), amino acid analogs β -alanine (β -Ala) and 2-aminobutyric acid (Abu), the dipeptide glycylglycine (GlyGly), as well as common hydrophilic and hydrophobic amines (hexylamine, pentylamine, benzylamine, isopropylamine, and di(ethylene glycol) methyl ether (DEG) amine). Hydrophobically modified acrylamide copolymers were prepared by reaction of pPFPA with ammonia in combination with benzylamine. Chemical structures of all amines are given in Scheme 1; Table 1 gives an overview of the (co)polymers prepared. Aminolysis of activated PFP ester side groups can be expected to cleave the trithiocarbonate end groups, and is, in fact, the most common way for effecting RAFT end group modification.[33, 34] In order to prevent side reactions of the resulting thiol end groups, acrylamide was added as a thiol scavenger (Michael acceptor), which, featuring an amide moiety, produces end groups with high chemical similarity to the (bis)amide repeating groups, thus eliminating possible end groups effects on aqueous solution behavior (see Scheme 1).

Successful reaction was confirmed by a combination of FT-IR and NMR spectroscopies, as well as size exclusion chromatography (SEC). Representative ^1H NMR spectra of homo- and copolymers are shown in Figure 1A and the supporting information. Spectra showed signals conforming to the structures expected for quantitative reaction of the PFP functional groups.

Molar compositions of copolymers were determined by comparison of the integrals of key resonances of each comonomer functionality and are listed in Table 1. ^{19}F NMR spectroscopy of reaction mixtures before purification showed signals characteristic of free pentafluorophenol and no remaining PFP ester signals, confirming full reaction of the PFP ester groups, see supporting information. A comparison of FT-IR spectra of bisamide product (co)polymers with a spectrum of a pPFPA precursor, shown in Figure 1B, indicated the complete disappearance of characteristic bands associated with the precursor (PFP ester $\text{C}=\text{O}$ stretching at 1780 cm^{-1} , $\text{C}-\text{F}$ stretching at 1520 cm^{-1} , and aromatic $\text{C}=\text{C}$ stretching—multiple weak bands from $1480\text{--}1440\text{ cm}^{-1}$) and the appearance of bands characteristic of secondary amides ($\text{N}-\text{H}$ stretching at $3500\text{--}3250\text{ cm}^{-1}$, $\text{C}=\text{O}$ stretching (amide I band) at $1720\text{--}1600\text{ cm}^{-1}$, $\text{N}-\text{H}$ bending (amide II band) at $1580\text{--}1500\text{ cm}^{-1}$) during the reaction, likewise confirming complete modification. SEC (of species soluble in dimethylacetamide) revealed product (co)polymers to have molar mass distributions similar to the parent precursors with no evidence of crosslinking or polymer decomposition, see supporting information and Table 1.



Scheme 1. Synthesis of homo- and copolymers from pPFPA with amines and structures of amines including abbreviations of analogous amino acids for amino acid amides

Table 1. Overview of (Co)Polymers prepared from pPFPA with Feed Ratio of Amines, their Molar Composition determined by NMR, Molar Mass Details and Solubility Behavior.

Entry	Name, Abbreviation	precursor	Amine A (feed ratio in %)	Amine B (feed ratio in %)	$M_{n,theor.}^a$ [kg/mol]	$M_{n,SEC}^b$ [kg/mol]	\bar{D}_M^b	Behavior (CP in °C)
1	Poly(<i>N</i> -acryloyl glycinamide), pGlyAm	pPFPA1	1 (100%)		13.4			S
2	Poly(<i>N</i> -acryloyl alaninamide), pAlaAm	pPFPA1	2 (100%)		14.8	19.6	1.40	I
3	Poly(<i>N</i> -acryloyl alaninamide), pAlaAm	pPFPA2	2 (100%)		5.8	8.9	1.41	I
4	Poly(<i>N</i> -acryloyl serinamide), pSerAm	pPFPA1	3 (100%)		16.5	37.7	1.27	S
5	Poly(<i>N</i> -acryloyl aminobutyramide), pAbuAm	pPFPA1	4 (100%)		16.3	45.0	1.37	S
6	Poly(<i>N</i> -acryloyl valinamide), pValAm	pPFPA1	5 (100%)		17.7	31.7	1.48	I
7	Poly(<i>N</i> -acryloyl leucinamide), pLeuAm	pPFPA1	6 (100%)		19.1			I
8	Poly(<i>N</i> -acryloyl β -alaninamide), p(β -AlaAm)	pPFPA1	7 (100%)		14.8			S
9	Poly(<i>N</i> -acryloyl glycylglycine), pGlyGlyAm	pPFPA1	8 (100%)		19.3			S
10	Poly(<i>N</i> -acryloyl glycinamide _{0.75} -co- <i>N</i> -benzylacrylamide _{0.25}), p(GlyAm _{0.75} -co-BzAm _{0.25})	pPFPA1	1 (90%)	10 (10%)	14.2	33.7	1.31	I
11	Poly(<i>N</i> -acryloyl glycinamide _{0.70} -co- <i>N</i> -hexylacrylamide _{0.30}), p(GlyAm _{0.70} -co-HexAm _{0.30})	pPFPA1	1 (90%)	11 (10%)	14.2	37.5	1.46	I
12	Poly(<i>N</i> -acryloyl aminobutyramide _{0.74} -co- <i>N</i> -benzylacrylamide _{0.26}), p(AbuAm _{0.74} -co-BzAm _{0.26})	pPFPA1	4 (90%)	10 (10%)	16.4	44.6	1.31	LCST (30.2)
13	Poly(<i>N</i> -acryloyl aminobutyramide _{0.76} -co- <i>N</i> -acryloyl leucinamide _{0.24}), p(AbuAm _{0.76} -co-LeuAm _{0.24})	pPFPA1	4 (75%)	6 (25%)	17.0	47.1	1.24	S
14	Poly(<i>N</i> -acryloyl aminobutyramide _{0.55} -co- <i>N</i> -acryloyl leucinamide _{0.45}), p(AbuAm _{0.55} -co-LeuAm _{0.45})	pPFPA1	4 (50%)	6 (50%)	17.6	40.2	1.48	LCST (43.8)
15	Poly(<i>N</i> -acryloyl aminobutyramide _{0.29} -co- <i>N</i> -acryloyl leucinamide _{0.71}), p(AbuAm _{0.29} -co-LeuAm _{0.71})	pPFPA1	4 (25%)	6 (75%)	18.3	43.5	1.40	I
16	Poly(<i>N</i> -acryloyl valinamide _{0.67} -co- <i>N</i> -diethylene glycol acrylamide _{0.33}), p(ValAm _{0.67} -co-DEGAm _{0.33})	pPFPA1	5 (90%)	12 (10%)	17.8			LCST (31.8)
17	Poly(<i>N</i> -acryloyl alaninamide _{0.73} -co- <i>N</i> -pentylacrylamide _{0.27}), p(AlaAm _{0.73} -co-PentAm _{0.27})	pPFPA2	2 (80%)	13 (20%)	5.8	10.3	1.27	I
18	Poly(<i>N</i> -acryloyl alaninamide _{0.78} -co- <i>N</i> -diethylene glycol acrylamide _{0.22}), p(AlaAm _{0.78} -co-DEGAm _{0.22})	pPFPA2	2 (80%)	12 (20%)	6.0	11.1	1.27	S
19	Poly(<i>N</i> -acryloyl alaninamide _{0.46} -co- <i>N</i> -acryloyl glycinamide _{0.54}), p(AlaAm _{0.46} -co-GlyAm _{0.54})	pPFPA1	2 (50%)	1 (50%)	14.0			S
20	Poly(<i>N</i> -acryloyl alaninamide _{0.38} -co- <i>N</i> -isopropylacrylamide _{0.62}), p(AlaAm _{0.38} -co-IPAm _{0.62})	pPFPA1	2 (20%)	14 (80%)	13.0	62.1	1.29	LCST (50.9)
21	Poly(acrylamide), pAm	pPFPA1	9 (100%)		7.5			S
22	Poly(acrylamide _{0.94} -co- <i>N</i> -benzylacrylamide _{0.06}), p(Am _{0.94} -co-BzAm _{0.06})	pPFPA1	9 (95%)	10 (5%)	8.1			S
23	Poly(acrylamide _{0.87} -co- <i>N</i> -benzylacrylamide _{0.13}), p(Am _{0.87} -co-BzAm _{0.13})	pPFPA1	9 (90%)	10 (10%)	8.7			UCST (7.1)
24	Poly(acrylamide _{0.81} -co- <i>N</i> -benzylacrylamide _{0.19}), p(Am _{0.81} -co-BzAm _{0.19})	pPFPA1	9 (85%)	10 (15%)	9.3			I
25	Poly(acrylamide _{0.69} -co- <i>N</i> -benzylacrylamide _{0.31}), p(Am _{0.69} -co-BzAm _{0.31})	pPFPA1	9 (80%)	10 (20%)	10.4			I

^a Molar mass of (co)polymers calculated from degree of polymerization of precursor and copolymer composition from NMR

^b Polystyrene equivalent molecular weight and dispersity (D_M) determined by SEC.

^c Solubility in water (concentration 5 g/L, 0–90 °C) S = soluble, I = insoluble; CP = cloud point determined at 50% transmittance

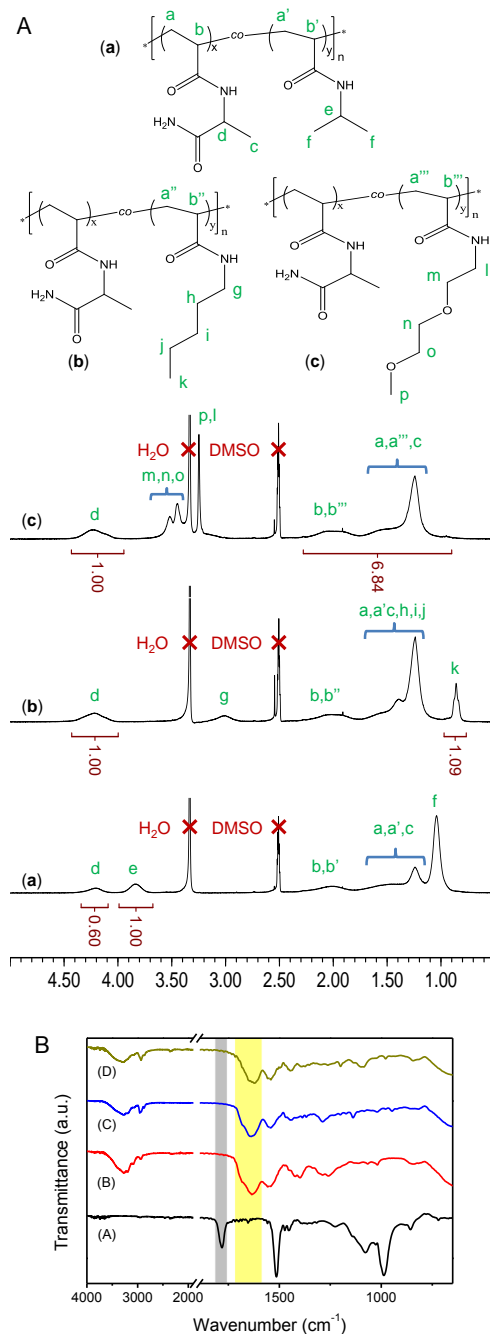


Figure 1. **A)** ¹H NMR spectra measured in DMSO-d₆ of the Ala-containing copolymers p(AlaAm_{0.38}-co-IPAm_{0.62}) (a); p(AlaAm_{0.73}-co-PentAm_{0.27}) (b); and p(AlaAm_{0.78}-co-DEGAm_{0.22}) (c) with peak assignments. **B)** FT-IR spectra of the pPFPA precursor (A), pGlyAm (B), pLeuAm (C), and p(ValAm_{0.67}-co-DEGAm_{0.33}) (D). The characteristic bands of the activated ester C=O stretch (gray) and the amide C=O stretch (yellow) are marked.

Aqueous Solution Behavior

With a library of novel (bis)amide (co)polymers prepared by postpolymerization modification of pPFPA in hand, we next investigated their aqueous solution behavior. pGlyAm (Table 1, entry 1) exhibited thermoreversible gelation in water at concentrations above 15 wt% (Figure 2AB), reversibly switching between a gelled and a free-flowing state, as described previously for this species,[2] and confirming successful synthesis of a thermoresponsive species through postmodification of pPFPA. The gel–sol transition of pGlyAm at a concentration of 30 wt% (photograph in Figure 2AB) occurred at 75 °C; gelation upon cooling occurred at 69°C, exhibiting a hysteresis of 6 °C. Two further (co)polymer samples, p(AlaAm_{0.46}-co-GlyAm_{0.54}) (entry 19) and pSerAm (entry 4), at concentrations above 30 wt%, also formed gels in water which melted upon heating (79 °C for p(AlaAm_{0.46}-co-GlyAm_{0.54}), 80 °C for pSerAm) forming a more viscous, ‘honey-like’ liquid. Upon cooling, gelation occurred with a hysteresis of 8 °C indicating thermoreversible behavior.



Figure 2. A) Photographs of (left to right) pGlyAm, pSerAm, and p(AlaAm_{0.46}-co-GlyAm_{0.54}) gels in water (30 wt-%); B) Photograph of same pGlyAm sample after heating.

The yellow color is due to RAFT agent end groups.

In order to identify species with thermoresponsive behavior in more dilute solution, (co)polymers were tested for water solubility at a concentration of 5 g/L in the temperature range 0–90 °C; results are summarized in Table 1. Within the series of homopolymers (Table 1, entries 1–9) pGlyAm, p(β-AlaAm), pAbuAm (formally pGlyAm with pendant ethyl

chains), and pGlyGlyAm were water soluble while the more hydrophobic species pValAm and pLeuAm (isopropyl and isobutyl side chains) were insoluble. pGlyAm did not show UCST behavior, possibly due to acrylic acid contamination of the monomer or polymer through hydrolysis reactions.[10] Surprisingly, pAlaAm (entry 2, methyl side chains) was found to be insoluble, suggesting stronger inter- and intramolecular hydrogen bonding than in the Abu analogue and the isomeric β -Ala species. Insolubility of pAlaAm was also confirmed on a sample with a lower molecular weight ($M_n = 5.8$ kg/mol, entry 3) prepared from pPFPA2. With amino-acid based bisamide polymers receiving attention as (UCST-type) thermoresponsive materials, we investigated the possibility of realizing LCST transitions within this family of polymers by tuning their hydrophilic–hydrophobic balance,[35] which is easily achieved by postpolymerisation modification by employing a mixture of modifying reagents.[20, 21] First, we hydrophobically modified the water soluble species pGlyAm and pAbuAm. pGlyAm-based copolymers containing 25 mol% benzylacrylamide and 30 mol% hexylacrylamide (entries 10 and 11) were both insoluble in water. Containing 26 mol% benzylacrylamide p(AbuAm_{0.74-co}-BzAm_{0.26}) (entry 12), however, was found to show LCST behavior in water with a measured cloud point of 30 °C. In a series of copolymers of AbuAm and LeuAm, entries 13–15, p(AbuAm_{0.76-co}-LeuAm_{0.24}) (soluble), p(AbuAm_{0.55-co}-LeuAm_{0.45}) (LCST cloud point at 44 °C), and p(AbuAm_{0.29-co}-LeuAm_{0.71}) (insoluble) solubility decreased—expectedly—with increasing LeuAm content, showing successful design of an LCST-type copolymer consisting entirely of amino acid amide-derived monomer units. These observations confirm that (ethyl side chain) AbuAm segments are indeed hydrophilic (in spite of the methyl side chain analogous polymer pAlaAm being insoluble), since their incorporation into the hydrophobic pLeuAm species increased solubility and evoked LCST behavior. Of note, both AbuAm-based copolymers p(AbuAm_{0.55-co}-LeuAm_{0.45}) and p(AbuAm_{0.74-co}-BzAm_{0.26}) showed broad LCST transitions with transmittance decreasing gradually over a range of 15–20 °C, Figure 3A. LCST behavior

could also be induced by means of hydrophilic modification of the water insoluble species pValAm. Containing 33 mol% of DEG acrylamide comonomer, p(ValAm_{0.67}-co-DEGAm_{0.33}), entry 16, showed a relatively sharp cloud point at 32 °C, Figure 3A, suggesting that, irrespective of the bisamide functionality, tuning of aqueous solution behavior and realization of LCST transitions was possible. We next investigated pAlaAm-based polymers in more detail due to the unexpected insolubility of the pAlaAm homopolymer in water and prepared four copolymers, Table 1 entries 17–20. Their aqueous solution behavior appeared to be dictated by the nature of the comonomer. Containing 24 mol% pentylacrylamide, p(AlaAm_{0.73}-co-PentAm_{0.27}) was insoluble, while incorporation of 18 mol% DEG acrylamide comonomer resulted in full aqueous solubility. Likewise, a near-equimolar copolymer of Ala and Gly-based segments was soluble in water. Reaction of pPFPA with a mixture of isopropyl amine and alaninamide produced a pNIPAM-based copolymer containing 38 mol% Ala-units. This copolymer exhibited LCST behavior with an onset of transmittance decrease around 32 °C, the well-documented LCST of pNIPAM,[36] though with an extremely broad transition, apparently caused by the presence of the strong hydrogen bonding comonomer units. While (co)polymers exhibiting sharp thermal transitions are often considered ideal systems, we note that gradual transitions (for example those found for UCST transitions in water–alcohol mixtures) have specifically been exploited for sensing applications over a wider temperature range than would be possible with typical LCST systems.[37]

To the best of our knowledge, thermoresponsive (co)polymers derived through postmodification of activated ester precursors have only exhibited LCST-type transitions. Intending to also realize UCST transitions employing this method, we prepared a series of acrylamide–benzylacrylamide copolymers, Table 1 entries 21–25. With low amounts (0 and 6 mol%) of the hydrophobic modifier, the resulting (co)polymers were soluble in water, while a sample containing 13 mol% benzylacrylamide showed a gradual UCST transition in water

(Figure 3B). With higher contents of benzylacrylamide, however, polymers could not be dissolved in water, suggesting poor control over UCST tuning using this synthetic approach.

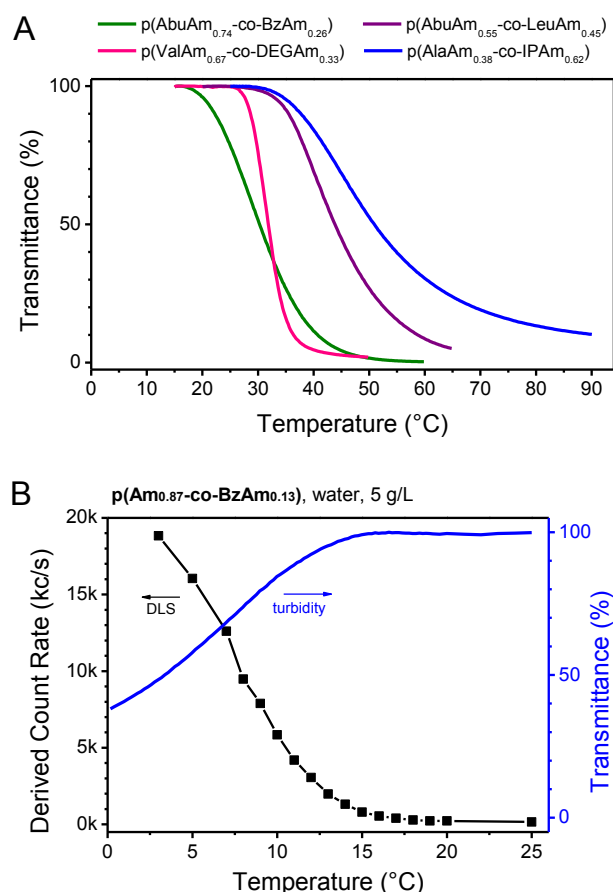


Figure 3. **A)** Transmittance curves of heating copolymer solutions showing LCST behavior in water (cooling curves shown in supporting information) **B)** Scattering intensity (by DLS, black curve) and transmittance (by turbidity, blue curve) of $p(\text{Am}_{0.87}\text{-co-BzAm}_{0.13})$ showing concurrent decrease of transmittance and increase of scattering count rate upon cooling indicating UCST behavior in water.

Self-Association

Apart from effecting thermoreversible attractions between polymer chains during gelation or UCST transitions in certain (co)polymer species, the strong hydrogen bonding capacity of amide groups plays an integral role in directed self-association, for example in the folding of proteins into quaternary structures or the helical structure of DNA. Inspired by nature, such attractions have been widely exploited in the supramolecular arena for the generation of 1D, 2D, and 3D highly ordered and hierarchical assemblies.[38, 39] While hydrogen bonding polymer–polymer attractions in aqueous solution of most (co)polymer species presented here were, apparently, not sufficient to evoke UCST transitions, we explored their ability to direct self-assembly into ordered structures in the dry state. Aqueous (co)polymer solutions (concentration 2 g/L) were freeze-dried and the morphology of the resulting solids was analyzed by scanning electron microscopy (SEM) after coating with a thin layer of chromium, Figure 4. Uniform, comparably large structures (tens to hundreds of micrometers in size), which differed dramatically with the chemical composition of the (co)polymers were observed. For example, pGlyAm self-associated into rod-like structures with diameters of 350–450 nm and lengths in excess of 35 μm (Figure 4 a,b). In contrast to pGlyAm, p(AlaAm_{0.46}-co-GlyAm_{0.54})—formally pGlyAm with 46 mol% stereoregular methyl-substitution—formed stacked sheets with edge lengths > 100 μm with worm-like outgrowths supporting the sheets at a distance of approximately 4.5 μm (Figure 4 c,d). A sheet-like morphology, though without worm-like outgrowths and an order of magnitude larger in size, with sheet lengths of several hundred micrometers, and sheet thicknesses of ~250 nm was also observed for pGlyGlyAm, see Figure 4 g,h. The LCST sample p(AbuAm_{0.74}-co-BzAm_{0.26}), on the other hand, self-associated into honeycomb-like structures with rectangular pores of ~2.5×15 μm sizes and wall thicknesses of ~200 nm, Figure 4 e,f. Such order and uniformity of observed structures is impressive, as is the strong influence of chemical structure on the observed morphology. Of note, a sample of the pNIPAM-based species p(AlaAm_{0.38}-co-

IPAm_{0.62}) did not assemble into uniform structures giving a mixture of sheets, rods, and spherical structures, suggesting a possible importance of bisamide moieties and, perhaps, stereoregularity throughout the polymer, see supporting information. Vesicular morphology of end-group modified polymers after freeze-drying polymer solutions has previously been described,[40, 41] though this process is not fully understood. We assume that structuring occurs during freeze-drying at the air–ice interface where a gradual evaporation of their hydration sphere allows (co)polymer chains a certain mobility (that may well be governed by the manner in which individual water molecules are removed from the backbone and the two amide side groups) which causes chains to self-associate in a particular fashion, affording a growth of regular structures. The direction in which the ice evaporated from the sample could give rise to the anisotropic character of the observed structures. With large interest in producing self-assembled regular structures, we believe that our observations offer great potential for applications requiring large surface areas, e.g. catalysis, or in the fabrication of membranes.

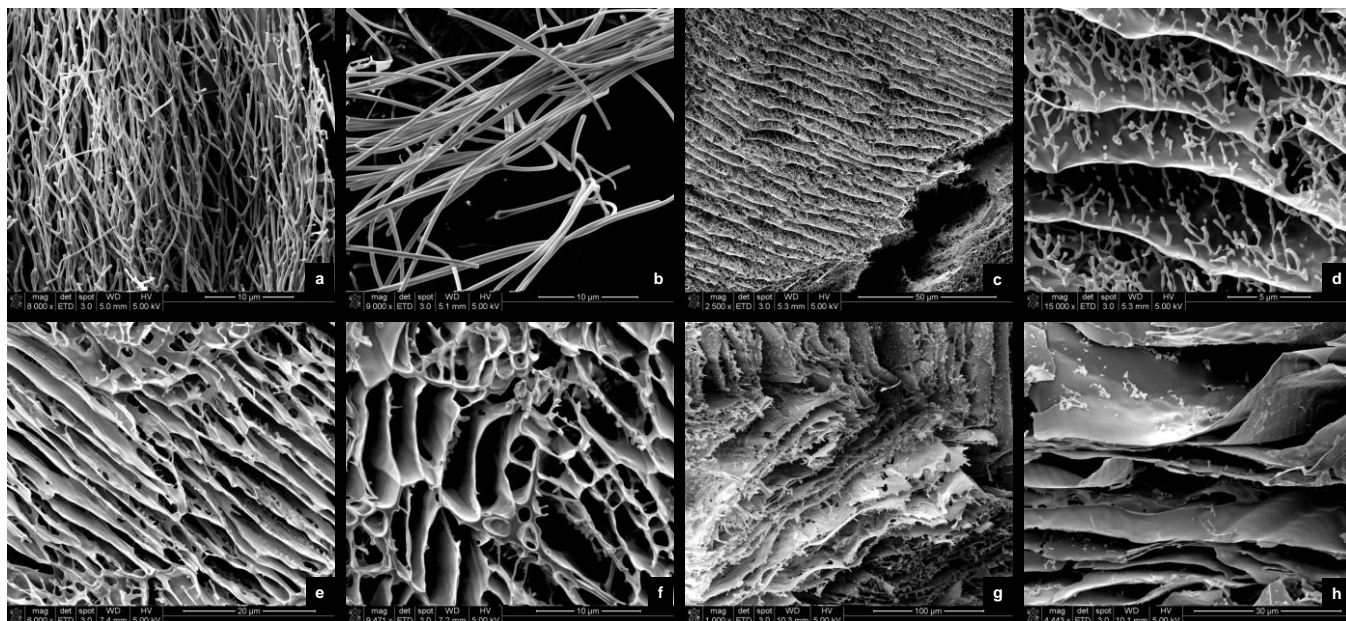


Figure 4. SEM images of dry pGlyAm (**a, b**; scale bars 10 μm), p(AlaAm_{0.46}-co-GlyAm_{0.54}) (**c, d**; s.b. 50 μm and 5 μm), p(AbuAm_{0.74}-co-BzAm_{0.26}) (**e, f**; s.b. 20 μm and 10 μm), and pGlyGlyAm (**g, h**; 100 μm and 30 μm)

Conclusions

Postpolymerization modification of activated ester precursors provided access to a library of novel, well-defined bisamide (co)polymers and hydrophobically modified acrylamide copolymers. While UCST behavior, as described in the literature for several of such polymeric materials, was only found for one sample, poly(acrylamide_{0.87}-co-*N*-benzylacrylamide_{0.13}), several bisamide copolymers were designed to show LCST behavior exhibiting very broad transitions. Interestingly, (co)polymers self-associated into highly regular, ordered structures upon freeze-drying, a promising phenomenon that we are currently investigating in more detail.

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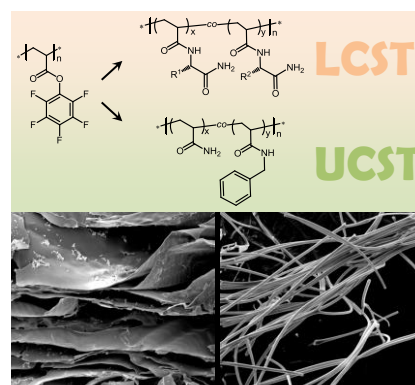
Keywords: phase behavior, self-organization, gelation, thermoresponsive, postpolymerization modification

Graphical Abstract

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Postpolymerization Synthesis of (Bis)Amide (Co)Polymers: Thermoresponsive Behavior and Self-association

Governed by strong intra- and intermolecular hydrogen bonding between amide groups, a series of novel (bis)amide (co)polymers prepared by post-polymerization modification of an activated ester scaffold is shown to reversibly gel in water at low temperatures, display LCST or UCST behavior in water and to self-organize into large sheet-like or rod-like structures.



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